RHOA-regulated IGFBP2 promotes invasion and drives progression of BCR-ABL1 chronic myeloid leukemia

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Summary of Antibodies used in this study.

Antibodies used for western blotting were (dilution 1:1000): β-Actin (Cell Signaling, #5125), RHOA (Cell Signaling, #2117), IGFBP2 (Cell Signaling, #3922), IL20RB (ABclonal, A7980), CD24 (Santa Cruz, sc-19585), GAPDH (Santa Cruz, sc-25778), Lamin A (Santa Cruz, sc-518013), SRF (Cell Signaling, #5147).

Design of sgRNAs used in this study.

For locus-specific deletion of RHOA in CML cells, we designed two 20 nucleotides (nt) guide sgRNAs targeting the 5' and 3' flanking regions of the gene (sgRNA1: 5'-*AATCACCAGTTTCTTCCGGA* -3'; sgRNA2: 5'- *CTGCTCTGCAAGCTAGACGT* -3'), using CRISPR Targets Track on Genome Browser. Two additional sgRNAs, sgRNA3: 5'-*AGTCTGGGTCCGTTCCACGC* -3' and sgRNA4: 5'- *GTGAACATTTGACTCGACGG* -3' were used for generation of IGFBP2 knockout clones. The mock control clones were generated using the non-targeting sgRNA sequence 5'- AAAUGUGAGAUCAGAGUAAU -3' (Thermo Fisher Scientific).

Summary of primers used in CHIP analyses.

ChIP-qPCR primers P1 were designed to amplify a proximal promoter region containing two putative serum response elements (SREs) (5'- CTCCAAAAGGGGGA -3' and 5'-GCCCTTTAGGACCCG-3'), with P1 forward primer 5'- GAAGAGTGCGGAGGGACG -3' and P1 reverse primer 5'- GGTCCTAAAGGGCCGGCTT -3'. Another primer pair, P2, targeting an intronic region, was selected as a negative control, with P2 forward primer 5'-GTCCTTGGGGAGACAGAACG -3' and P2 reverse primer 5'-AGGCCTGAGAACTGAAAGCC -3'